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ENHANCING THE LYCOPENE *IN VITRO* BIOACCESSIBILITY OF TOMATO JUICE SYNERGISTICALLY APPLYING THERMAL AND NON-THERMAL PROCESSING TECHNOLOGIES

K.G.L.R. Jayathunge[a], Alexandros Ch. Stratakos[a], Oliver Cregenzán-Albertia[b], Irene R. Grant[a], James Lyng[b], Anastasios Koidis[a]

[a] Institute for Global Food Security, Queen's University Belfast, Belfast, Northern Ireland, UK.

[b] School of Agriculture and Food Science, University College Dublin, Ireland.

Corresponding author:

Dr Anastasios (Tassos) Koidis, Institute for Global Food Security, Queen's University Belfast, 18-30 Malone Road, Belfast, BT9 5BN, Northern Ireland, UK, Tel: +44 28 90975569 email: t.koidis@qub.ac.uk

ABSTRACT

The influence of moderate intensity pulsed electric field pre-processing on increasing the lycopene bioaccessibility of tomato fruit, and the combined effect of blanching, ultrasonic and high intensity pulsed electric field processing on further enhancement of the lycopene bioaccessibility after juicing were investigated. Maximum total lycopene bioaccessibility (9.6%) of the tomato fruit was achieved by a 4 µs pre-processed treatment after 24 h holding period and further processing results revealed that all treatments were effective to increase the total lycopene. Most of juice processing treatments decreased the release of lycopene from the tomato matrix during digestion. Only the treatment of blanching followed by high intensity pulsed electric field showed a significant release of *trans*- (4.01±0.48) and *cis*- (5.04±0.26 µg/g) lycopene, achieving 15.6% total lycopene bioaccessibility. Thus, processing of pre-blanched juice using high intensity pulsed electric field, derived from pre-processed tomato was the best overall process to achieve the highest nutritive value.

Key words; Tomato; juice; blanching; lycopene; *in vitro* bioaccessibility; pulsed electric fields; ultrasonic; processing

1. INTRODUCTION

Sufficient uptake of lycopene from the diet is necessary to benefit from its health promoting effects, since humans are unable to synthesise lycopene *de novo*. Low lycopene bioaccessibility/bioavailability is an identified worldwide issue; therefore there is a focused interest in ways of increasing the uptake of lycopene from the human diet. One of the ways to do this is through a diet rich in tomato juice. It is a real challenge to develop processing conditions during tomato juice production that can result in maximum overall lycopene bioaccessibility.

Different non-thermal processing technologies have emerged in the last few decades to fulfil the consumer desire for highly nutritious "fresh-like" tomato juice products that could potentially replace traditional thermal processing. Among them, pulsed electric fields (PEF) and ultrasonication (US) techniques have been widely investigated (Adekunte, Tiwari, Scannell, & O'Donnel, 2010; Mosqueda-Melgar, Raybaudi-Massilia, & Martín-Belloso, 2012; Nguyen and Mittal, 2007; Odriozola-Serrano, Aguilo-Aguayo, Soliva-Fortuny, Gimeno-Ario, & Martín-Belloso, 2007; Odriozola-Serrano, Solivia-Fortuny, Hernandez-Jover, & Martin-Belloso, 2009). Most non-thermal processing studies have focused on the retention of lycopene content after processing, which is not necessarily representative of its biological value because it is not indicative of how much is bioavailable after ingestion (Krebbers, Matser, Hoogerwerf, Morzelaar, Momassen, & Van den berg, 2003; Odriozola-Serrano et al., 2007). In most of these studies, the impact of PEF and US processing on lycopene bioaccessibility of tomato juice has not been well characterised. Anese, Mirolo, Beraldo & Lippe (2013) studied the effect of ultrasonic treatments on the microstructure of tomato pulp and lycopene *in vitro* bioaccessibility and observed a more than three-fold reduction in lycopene bioaccessibility after ultrasonication. No systematic studies, neither *in vivo* nor *in vitro*, that report lycopene bioaccessibility in tomato

as a function of a range of different non-thermal processing technologies in comparison to thermal processing techniques seem to exist currently.

There has been increasing interest in the use of moderate intensity pulsed electric field (MIPEF) technology due to its potential to induce non-thermal cell permeability and stress reaction at the cellular level in the plant (Soliva-Fortuny, Balasa, Knorr, & Martin-Belloso, 2009). These cell permeability and stress reactions have been reported to be beneficial in enhancing and stimulating the polyphenolic and carotenoid content in plants (Balasa, Toepfl, & Knorr, 2006; Toepfl, Mathys, Heinz, & Knorr, 2006; Vallverdu-Queralt, Oms-Oliu, Odriozola-Serrano, Lamuela-Raventos, Martin-Belloso, & Elez-Martinez, 2013b). The effect of MIPEF on lycopene bioaccessibility of whole tomato fruits and their product, such as tomato juice, is however largely unknown. The concept of using MIPEF technology as a pre-processing treatment in advance of other processing techniques to enhance lycopene bioaccessibility is also under explored, and successful implementation of such a pre-treatment could result in products with an additional advantage with regards to nutritive value.

Hence, the aims of this study were two fold. Firstly, to investigate the feasibility of MIPEF as a pre-treatment for the whole tomato fruit in order to enhance the lycopene *in vitro* bioaccessibility. Secondly, to further enhance lycopene bioaccessibility of tomato juice products by evaluating the best combination of conventional and novel processing technologies.

2. MATERIALS AND METHODS

2.1 Materials

Tomato fruits of *Pitenza* (origin- Spain) variety at fully ripe stage were purchased in several batches from wholesalers in Northern Ireland (UK) between December 2014 and October 2015. In total, 60 kg of tomatoes were purchased in six batches and graded before processing (mean

weight, 85±5 g and mean circumference, 15±1 cm); odd shaped and sized tomatoes were excluded. The *all-trans* and 5-*cis* lycopene standards were purchased from CaroteNature (Lupsingen, Switzerland) and all other chemicals were purchased from Sigma-Aldrich (Dorset, UK). All chemicals were of analytical grade. Packaging materials (polyethylene/polyamide film, 200 µm thickness) were obtained from Scobie & Junor (Mallusk, UK).

2.2 Pre-treatment: optimisation of MIPEF in the whole tomato fruit

Moderate intensity pulsed electric field (MIPEF) treatments were conducted in batch mode using a laboratory-scale PEF unit (ELCRACK HVP 5, DIL, German Institute of Food Technologies, Quackenbruck, Germany) located within the School of Agriculture and Food Science, University College Dublin. A stainless steel parallel treatment chamber with 8 cm gap was used, and tomato fruit was placed in the treatment chamber and filled with tap water. Whole ripe tomatoes were subjected to 0, 1, 20 or 80 mono-polar pulses (pulse width- 4 µs, frequency- 0.1 Hz, electrical field strength (EFS)- 1 kV/cm), which is equivalent to 0, 4, 80 and 320 µs treatment duration, respectively. MIPEF-treated and untreated fruits were collected and to investigate the potential to induce stress reactions in plant systems in production of secondary metabolites, fruits were immediately refrigerated at 4 ℃ and held for 48 h. Each treatment consisted of three replicates with six fruits per replicate, and the whole experiment was conducted on two separate days as independent batches. Samples were withdrawn after 0, 24 and 48 h holding period (dark storage) and subjected to analysis. Samples used to measure lycopene *in vitro* bioaccessibility were stored at -80 ℃, while the rest of the samples were refrigerated (4 ℃) for microstructure analysis.

2.3 Processing of tomato juice

2.3.1 Preparation of tomato juice for processing

Tomatoes were subjected to MIPEF treatment (1 pulse, 4 µs pulse width, 1 kV/cm EFS, 0.1 Hz) and refrigerated immediately after the treatment at 4℃ for 24 h. Untreated tomatoes as a control treatment were also refrigerated at 4 ℃. After 24 h holding at 4 ℃, untreated and MIPEF-treated tomatoes were separately blended (120 s at 10000 rpm) using a domestic blender and filtered through 2 mm steel sieve and subjected to processing as described below.

2.3.2 Thermal and non-thermal processing of tomato juice

In order to investigate the contribution of each processing step to increasing the lycopene *in vitro* bioaccessibility, starting from fresh tomato to processed juice, the experiment was conducted with six treatments (fresh, MIPEF, MIPEF+blanching (B), MIPEF+B+US, MIPEF+B+HIPEF and MIPEF+B+US+HIPEF) and each treatment consisted of three replicates. The overall experimental design, representing both pre-treatment and processing experiments, is depicted in Figure 1. Blanching (90 ℃/2 min) step was conducted using a water bath (Grant OLS 200, Wiltshire, UK) after filling the juice (50 ml) into heat sealed pouches (10x10 cm). The juice temperature was monitored using K-type thermocouples fitted to a data logger (Grant squirrel 2040 series, Wiltshire, UK). Blanched samples were cooled immediately using an ice bath and then subjected to US and HIPEF processing. US processing was conducted using a 400 W ultrasonic processor (Model No. UIP 1000 hd, Hielscher, Germany) with a 19 mm diameter probe. Samples (50 ml) were processed at a constant frequency of 20 kHz and 20% amplitude level for 7 min according to Adekunte et al. (2010). Tomato juice samples were placed in a 50 ml beaker and juice temperature during sonication increased to 65-70 ℃. The ultrasonic probe was submerged to a depth of 25 mm in the sample.

HIPEF treatments were also conducted in batch mode using a laboratory scale PEF unit (ELCRACK HVP 5, DIL, German Institute of Food Technologies, Quackenbruck, Germany) located within the School of Agriculture and Food Science, University College Dublin. A stainless steel parallel treatment chamber with 8 cm gap was used to process 50 ml of juice. Treatment was set up at 35 kV/cm for 1500 μs using 4 μs width pulses and a frequency of 100 Hz (Odriozola-Serrano et al., 2009). As a control, heat sealed pouches of the same tomato juice were subjected to thermal processing (95 °C/20 min) using a water bath. The *cis* and *trans* lycopene contents, lycopene *in vitro* bioaccessibility, and colour of the all processed samples were evaluated as per methods described below.

2.4 Physical and chemical analysis of whole tomato and tomato juice

2.4.1 Microstructure

Scanning electron microscopy (SEM) was employed to evaluate the impact of processing on the microstructure of tomato. Small pieces of mesocarp of treated and untreated tomatoes were taken from the outer mesocarp close to the skin. All pieces were cut with a scalpel to sizes of approximately 0.5 x 2.0 x 2.0 mm, immediately placed in 5% glutaraldehyde (in 0.1 M sodium phosphate buffer, pH 7.4) for 24 h at 4 °C, and then dipped three times (20 min each) in 0.1 M sodium phosphate buffer, three times (20 min each) in double-distilled water, and dehydrated in a series of ethanol (50-100%) solutions. Additionally, samples were dipped in hexamethyldisilazane (HMDS) solution for 20 min and then dried under a fume hood overnight. Before taking the microstructure images, dried tissue samples were fixed on steel supports and coated with gold using an Emitech metaliser (Quorum Technologies, K575 X, Kent, UK) at

1100-1200 V, 5 mA for 10 min. Samples were observed using a scanning electron microscope (FEI, Qunta3D FEG, Oregon, USA) at 20 kV.

2.4.2 Determination of lycopene in vitro bioaccessibility

Tomato samples (2.5 g) were subjected to a simulated human gastric and small intestinal digestion based on the method described by Hedren, Mulokozi & Svanberg (2002) with a few modifications (Anese et al., 2013; Stratakos, Delgado-Pando, Linton, Patterson & Koidis, 2016) to determine the *in vitro* bioaccessibility of lycopene. Amber tubes and vials were used at all steps to protect samples from light. The digests were centrifuged (SORVALL Legend RT, Woburn, Germany) at 5000 g for 1 h to remove the non-digested particles (Failla, Huo, & Thakkar, 2008). The lycopene content in the supernatant was measured using the method described below (2.4.3). The lycopene bioaccessibility of a sample is reported as the ratio (%) of the *in vitro* bioaccessible lycopene content to the corresponding lycopene content of the sample. The simple static *in vitro* gastro- intestinal track (GIT) model used in this study cannot simulate the complex conditions that occur within the human gastro-intestinal tract. However, the approach of using digestion models is still useful in terms of rapid evaluation of nutrient bioaccessibility in foods, which subsequently can be investigated in depth using animal or human trials.

2.4.3 Determination of cis- and trans-lycopene isomers using high performance liquid chromatography (HPLC)

Lycopene was extracted from both undigested sample and digesta (content resulting after digestion) of whole tomato and tomato juice according to the method described by Sadler, Davis & Dezman (1990) with minor modifications. Lycopene extraction from undigested whole tomato

and tomato juice was carried out using freeze dried samples (0.25 g) and centrifuged digesta (15 ml) obtained after digestion with 25 ml of hexane:ethanol:acetone (2:1:1). Freeze drying was achieved using a Christ-Alpha 1-4 LD plus freeze dryer (Darmstadt, Germany). The apolar phase, containing lycopene, was separated from the polar phase using a separation funnel and concentrated under vacuum using a rotary evaporator (Stuart RE 3008, Staffordshire, UK) at 30 ^{o}C, and subsequently reconstituted in 2 ml hexane:dicholoromethane (4:1), filtered (Fisher PTFE filters, 0.20 µm pore size, 25 mm diameter) and transferred to a 2 ml amber vial.

For the analysis of lycopene isomers, an HPLC system (Waters Limited, 2695 series, Hertfordshire, UK) equipped with a diode array detector (DAD) was used. The different isomers were separated on a reversed phase C30 column (5 µm x 150 mm x 2.0 mm, Develosil, Macclesfield, UK). The column was thermostated at 25 ^{o}C. The initial mobile phase consisted of water (A), methanol (B) and methyl-tert-butyl-ether (C). The following gradient program was used: linear change of 4% A, 81% B and 15% C for 5 min, followed by 4% A, 36% B and 60% C for next 5 min and then switched to the initial conditions of 4% A, 81% B and 15% C, over another 10 min.

All-trans and different *cis*- lycopene isomers were identified based on retention time and previously reported spectral characteristics (Re, Fraser, Long, Bramley & Rice-Evans, 2001; Yeum, Booth, Sadowski, Liu, Tang & Ang Krinsky, 1996). For the quantification of lycopene, HPLC-DAD peak responses were measured at 472 nm and appropriate calibration curves were generated from peak areas of *all-trans* and *5-cis* lycopene standards. Since no standards were available for other *cis*-lycopene isomers, they were quantified based on the method of Colle, Lemmes, Van Buggenhout, Van Loey & Hendrickx (2010b) and expressed as total *cis*-lycopene. The lycopene isomer contents of tomato juice were calculated on a fresh weight basis, i.e. µg/g.

2.4.4 Colour measurements

The colour of tomato juice samples was evaluated using a Konica Minolta portable colorimeter (CR-400, Konica Minolta, Japan). The instrument was calibrated using a white standard tile (X= 00.31, Y=93.00, Z=0.3324). The tomato juice samples were placed in a transparent cup and the colorimeter was placed on the juice and L, a, b values were directly taken from the colorimeter. Each measurement reported represents the average of three readings. L represents lightness, +a represents redness, -a represents greenness, +b yellowness and –b represents blueness. The Hue value (a/b) was calculated based on measured a and b values.

2.4.5 Statistical analysis

Significance of the results and statistical differences were analysed using the SPSS (IBM, New York) statistical package. Data were analysed by two-way analysis of variance (ANOVA) procedure. Duncan multiple range test (DNMRT) was employed to determine the differences among treatment means, with $p<0.05$ considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 MIPEF as a pre-treatment in whole tomatoes

3.1.1 Effect of MIPEF treatment parameters on lycopene content of whole tomatoes

The contents of total lycopene, *all-trans* and *cis*-lycopene isomers of different treatments, before and after digestion (digesta), during a 48 h holding period are shown in Table 1. The fresh sample showed the lowest lycopene content (30.68±0.81µg/g FW) at zero holding time without MIPEF treatment , which was in accordance with the value described in the literature (Svelander, Tiback, Ahrne, Langton, Svanberg & Alminger, 2010). The level of *trans*-lycopene isomers of

the fresh samples (84.6%) are also in line with those described by other authors, which report *trans*- lycopene content in fresh tomatoes varied from 35-96% of total lycopene with 5-, 9-, 13- and 15-*cis* lycopene being the main *cis*- isomers detected (Frohlich, Conrad, Schmid, Breithaupt & Bohm, 2007). In addition to the dominant *all-trans* and the two *cis*-lycopene isomers, β-carotene could also be detected in the extracts (data not shown).

Both *trans*- and *cis*- isomers content in fresh tomatoes remained stable during 48 h holding period without significant differences at $p<0.05$ while enhancement of these isomers were observed in 4 and 80 µs treated fruits, except the *cis*- content in 4 µs treatment. The highest duration treatment (320 µs) showed the best lycopene enhancement immediately after the treatment (*trans*-lycopene 50.57±3.77 µg/g FW and *cis*-lycopene 7.41±0.69 µg/g FW) but decreased thereafter. The behaviour of *trans*- and *cis*- lycopene isomers in response to MIPEF duration and holding period in digesta, as calculated by the amount present in digesta, is shown in Table 1 and followed a similar pattern to the undigested samples of corresponding treatment. However, the *trans*- isomers content available in digesta was significantly reduced in all treatments (0.87±0.11-2.13±0.24 µg/g FW) in comparison to the amount present in undigested samples (24.84±2.08-50.57±3.77 µg/g FW). The *cis*- content of 4 and 80 µs treated samples increased 24 h after the treatment and decreased thereafter, while 320 µs treated sample showed continuous decreases. Similar decreases of *cis*- and *trans*-lycopene contents were reported by Valliverdu-Queralt, Odriozola-Serrano, Oms-Oliu, Lamuela-Raventos, Elez-Martinez & Martin-Belloso (2013a) for MIPEF treated (2 kV/cm, 120 µs) tomato 24 h after the treatment.

According to the literature, application of PEF to biological cell material makes the cell membrane permeable, which has proven effective in improving the polyphenol and pigments extractability without affecting other quality attributes of fruits and vegetables (Balasa et al.,

2006; Vallverdu-Queralt et al., 2013b). The external electrical field, in the form of short repeated pulses (μs or ms) of a high voltage (kV), induces either temporary (reversible) or permanent (irreversible) pores in the cell membrane. The reversible cell permeability is reported to be useful in inducing stress reactions in plant systems and stimulates the generation of secondary cell metabolites (Galindo, Dejmek, Lundgren, Rasmusson, Vicente & Moritz, 2009; Guderjan, Toepfl, Angersbach & Knorr, 2005; Toepfl et al., 2006) and consequently synthesis of carotenoids in plants as a defence response to stress (Balasa et al., 2006; Vallverdú-Queralt, Medina-Remon, Casals-Ribes, Andres-Lacueva, Waterhouse & Lamuela-Raventos, 2012). Therefore, enhancement of lycopene content immediately after the treatment and further increase 24 h after the treatment could be explained due to the cell membrane permeability and lycopene synthesis as a response to PEF treatment. Subsequent reduction after a 48 h holding period might be due to the reversible nature of the tissue permeability after some time period (Soliva-Fortuny et al., 2009).

3.1.2 Effect of MIPEF treatment parameters on microstructure of whole tomatoes

The results obtained from the quantification of *cis*- and *trans*- lycopene contents were supported by the microstructural changes observed in microscopic images taken from the SEM. Fresh or untreated tomato at 0 h, showed isodiametric parenchyma cells (Figure 2A), and this cellular structure of fresh samples remained stable throughout the holding period. With increasing treatment duration (80 and 320 μs), fruits show progressively more irregular cell wall structure, such as folds in cell walls and loss of smoothness (Supplementary material), unlike in fresh samples. Tomato fruits subjected to 80 μs, clearly showed the folds in the cell wall 24 h post-treatment (Supplementary material) and no folds can be seen at 48 h post-treatment

(Supplementary material). This might suggest the reversible nature of the cell wall structure as a result of MIPEF treatment, according to similar observations in the literature (Angersbach, Heinz & Knorr, 2000). The longest treatment (320 µs) showed the most dramatic changes, with more irregular and larger folds in the cell wall (Supplementary material) observed both immediately and 24 h after the treatment. The appearance of the cell wall 48 h post-treatment resembled a dried material (Supplementary material), which can be attributed to detrimental damage caused by high duration treatment. Hence, the microstructural differences observed during the holding period duration might be due to the previously reported pore formation phenomenon as a consequence of the MIPEF treatment (Angersbach et al., 2000) and this reversible cell permeability and irreversible breakdown of cell wall structure is expected to directly affect the micronutrient accessibility, not only of the tomato fruit but in general of all food producing plants (Soliva-Fortuny et al., 2009).

3.1.3 Effect of MIPEF treatment parameters on cis- and trans- lycopene bioaccessibility

Investigations of the *in vitro* bioaccessibility of lycopene from tomato are very limited, but values reported for the amount present in digesta are fairly low and in the range of 2-13 % of total content (Gartner, Stahl & Sies, 1997; Stahl & Sies, 1992; Svelander et al., 2010). Changes of *trans*- and *cis*- lycopene bioaccessibility as a response to MIPEF treatment duration and holding period are shown in Figure 3. Human studies have demonstrated the nutritional benefits of *cis*- isomers in tomato products, because these compounds seem to be better absorbed in the intestine than the *trans*- isomers (Takeoka, Dao, Flessa, Gillespie, Jewell & Huebner, 2001). Theresults in this study also support the findings of Takeoka et al. (2001), as *cis*-lycopene showed higher bioaccessibility (19-31%) in comparison to *trans*-lycopene bioaccessibility (2.38-

6.18%). The highest *trans*- (6.2%) and *cis*- (31%) lycopene bioaccessibilities were shown by the 4 µs treated samples immediately and 24 h after the treatment, respectively. Hence, the process of *trans*- to *cis*- isomerisation seems beneficial in terms of potential health impact of tomato juice, since the *cis*- isomers are more bioaccessible (approximately 5 times) in comparison to *trans*- isomers.

According to the results obtained from the MIPEF pre-treatment investigation, a treatment duration of 4 µs (one pulse at 0.1 Hz, 1 kv/cm) and a 24 h holding period were the most effective in enhancing total lycopene bioaccessibility among all treatments. This treatment (4 µs) showed 7.8% total lycopene bioaccessibility immediately after the treatment increasing up to 9.6% after 24 h holding period, and decreasing thereafter, reaching 8.1% after 48 h (Table 1).

3.2 Processing of tomato juice

3.2.1 Effect of thermal and non-thermal processing on lycopene content

The change in lycopene content of undigested samples and digesta during each processing step is shown in Table 2. Untreated juice contained 29.58±2.03 µg/g FW total lycopene content that consisted of 88% *all-trans* lycopene, which is in accordance with the results of Hart and Scott (1995) who reported that *all-trans* lycopene accounts for 56-91% of the total lycopene depending on the red tomato variety. Fresh tomato juice had 25.90±1.78 µg/g FW (87.6%) of *all-trans* and 3.68±0.03 µg/g FW (12.4%) *cis*- isomers content, while Colle et al. (2010b) claimed approximately three times higher *trans*- and *cis*- isomers contents in fresh tomato puree in comparison to our findings. Regarding the different treatment effects, *all-trans* lycopene retention was high in all cases; with dramatic changes in *cis*-lycopene content being observed for several different treatments. Enhancement of *cis*-lycopene content by 32.6, 4.89 and 151.9 %

was achieved by a single moderate intensity pulsed electric field treatment (MIPEF), MIPEF accompanied by blanching (MIPEF+B), and by a combination of moderate and high PEF treatment with intermediate blanching (MIPEF+B+ HIPEF), respectively, while reductions of 55.7, 22.5 and 23.3% were observed in MIPEF+B+US, MIPEF+B+US+HIPEF and TP samples, respectively. It has been reported that heat induced by blanching or HIPEF processing of juices may increase carotenoid level, including lycopene (Odriozola-Serrano et al., 2009; Vallverdu-Queralt et al., 2013b), due to activation of carotenoid isomerase enzymes and release of the carotenoids after removing the cellular barrier (Lemmens, Colle, Van Buggenhout, Palmero, Van Loey & Hendrickx, 2014; Van Buggenhout, Alminger, Lemmens, Colle, Knockaert & Moelants, 2010). Conflicting results regarding lycopene bioaccessibility and isomerisation during thermal processing have been reported, reflecting the complexity of cell behaviour during processing. Seybold, Frohlich, Otto & Bohm (2004) reported no lycopene isomerisation in typical tomato processing, while others (Boileau, Boileau & Erdman, 2002; Vallverdu-Queralt et al., 2013b) indicated the formation of *cis*-lycopene during thermal treatment of tomato puree and juice. The loss of *cis*- and *trans*- isomers due to degradation and oxidation during heat treatment have also been explored (Colle et al., 2010b; Svelander et al., 2010). Here, the different behaviour of *trans*- and *cis*- isomer lycopene content of tomato juice digesta is presented in comparison to the undigested counterpart (Table 2). Fresh tomato exhibited 1.30±0.02 µg/g FW of *trans*- and 1.08±0.16 µg/g FW of *cis*-lycopene content. *All-trans* lycopene content of MIPEF+B and TP samples, decreased by 64% while *cis*- lycopene content increased by 55 and 12%, respectively, in comparison to fresh samples. *All-trans* lycopene content after all other treatments increased significantly, with the highest content shown after the MIPEF+B+HIPEF treatment (4.01±0.02 µg/g FW). On the other hand, *cis*- isomer present in the digesta increased after all treatments,

with the highest content shown by the treatment of MIPEF+B+HIPEF (5.04±0.26 µg/g FW), with the exception of the MIPEF+B+US treatment. Boileau et al. (2002) also observed that more than 50% of carotenoids found in the human body are in the *cis*- isomeric form.

3.2.2 Effect of thermal and non-thermal processing on lycopene in vitro bioaccessibility

In addition to the lycopene isomer content, the *in vitro* bioaccessible lycopene content of the fresh and processed tomato juice was determined. This is reported as the ratio (%) of the lycopene isomer content in digesta to the lycopene isomer content in corresponding undigested treatment (Figure 4), which represents the percentage of the containing lycopene that is released from the tomato matrix during digestion. In the untreated tomato juice 5.02±0.09 % *trans*- and 46.95±4.35 % *cis*- lycopene isomers were bioaccessible, which is in contrast to Colle et al. (2010b) who reported 15% bioaccessibility in each isomer for untreated tomato pulp. Upon treatment, PEF+B+HIPEF and PEF+B+US+HIPEF were effective to enhance the *trans*-lycopene bioaccessibility significantly, while B, TP and B+US treatments showed lower *trans*-lycopene bioaccessibilities of 1.22, 1.49 and 3.85%, respectively, in comparison to fresh tomatoes. The bioaccessibility of *cis*-lycopene isomers in all treatments were comparatively high and within the range of 43% (TP) to 68% (MIPEF+B+US+HIPEF), agreeing with figures reported in the literature.

It has previously been reported that *cis*-lycopene isomers are less likely to crystallise or aggregate, and, therefore, they are thought to be more soluble in micelles (Boileau, Merchen, Wasson, Atkinson, & Erdman, 1999). In addition, studies in human and animal models support the hypothesis that *cis*- isomers are more efficiently digested (Boileau et al., 1999; Porrini, Riso, & Testolin, 1998; Stahl & Sies, 1992). However, the high bioaccessible nature of *cis*- isomers does not have significant impact when considering the total effect, since *trans*-lycopene

dominates in the processed samples (more than 84%) similar to fresh juice. Colle et al. (2010b) reported 25% *trans*- and 35% *cis*- biaccessibility after intense heat treatment (140 ^{o}C/30 min) of tomato pulp. Previous *in vivo* studies have shown that the absorption of lycopene from industrially processed (mechanically and thermally) tomatoes is higher than from fresh tomatoes (Gartner et al., 1997; Porrini et al., 1998). Considering total lycopene (both *cis*- and *trans*-) bioaccessibility, our results for TP samples (4.95%) are in very close agreement with the values reported by Svelander et al. (2010) and Ryan, O'Connell, O'Sullivan, Aherne & O'Brien (2008). An improvement in lycopene bioaccessibility after HTST blanching (90 ^{o}C/10 min) followed by boiling (100 ^{o}C/20 min) was reported by Svelander et al. (2010); however, the maximum bioaccessibility that they obtained was 29.3%, indicating that a large quantity of the lycopene was still bound to the food matrix.

In nature, lycopenes are tightly bound to subcellular lipids and binding proteins, which determine their release during digestion. During thermal processing proteins might form aggregates that are not available during digestion, hindering their subsequent incorporation into micelles, which is where lycopene might become embedded (Palmero, Lemments, Hendrickx & Loey, 2014). Takada and Nelson (1983) pointed out that upon heating, proteins in tomato denature, resulting in an irreversible complex formation. On the other hand, specific processing techniques can also induce new or additional barriers for carotenoid bioaccessibility; examples have been shown in the studies of Colle, Van Buggenhout, Van Loey & Hendrickx (2010a), Panozzo, Lemmens, Van Loey, Manzocco, Nicoli & Hendrickx (2013) and Svelander, Lopez-Sanchez, Pudney, Schumm & Alminger (2011) (high pressure homogenisation), and in the studies of Anese, Bot, Panozzo, Mirolo & Lippe (2015) and Anese et al. (2013) (ultrasound treatments). All these studies concluded that high pressure homogenisation or ultrasound treatments probably resulted in the

formation of a strong fibre network which hinders the release of lycopene from the tomato matrix in comparison to untreated tomato pulp. Interestingly, in the present study, *cis*- isomers bioaccessibility of US processed tomato juice was enhanced. This may be attributable to the MIPEF and blanching treatment applied prior to processing. Therefore, there is a strong case that pre- or post- treatment is needed for ultrasound processing in order to release the trapped lycopene during processing.

3.2.3 Effect of thermal and non-thermal processing on colour of tomato juice

Colour in the tomato is due to the presence of carotenoids, mainly lycopene; hence measuring colour is an indirect indicator of lycopene quantity present in a product. Colour properties as assessed by lightness (L value) and redness (a/b) of processed and unprocessed tomato juice were determined to investigate impact of processing method on sensory qualities directly after the treatment. By comparing the samples, marked colour differences were noticed (data not shown). Tomato juice obtained from fresh and MIPEF treated fruits showed the lowest redness indicated by the highest L values of 14.12±2.53 and 12.95±2.94, respectively. Colour was improved further by blanching and processing, showing lower L values in all processed samples ranging from 6.1-7.1. The colour of tomato juice is a direct reflection of lycopene content, but not necessarily related to the lycopene bioaccessibility. For instance, thermally processed samples showed lower bioaccessibility in comparison to other treated samples though it had higher redness (lower L value, 6.32±0.18).

4. CONCLUSIONS

The results of this study clearly demonstrate the benefit of MIPEF as a pre-processing treatment to enhance the lycopene bioaccessibility of whole tomato and further enhancement of lycopene bioaccessibility by subsequent processing. Total carotenoid and *cis*- and *trans*-lycopene contents could be enhanced by increasing the pre-treatment duration (from 4-320 µs, EFS-1kV/cm) to increase cell permeability. The lowest duration (4 µs) treatment showed the highest total lycopene bioaccessibility (9.6%) after a 24 h holding period. During subsequent tomato juice processing, total lycopene content increased after all treatments with the highest content (96.1%) shown by MIPEF+B+HIPEF treatment in comparison to fresh tomato juice. Results revealed that *cis*-lycopene was approximately five times more bioaccessible than *trans*-lycopene, and the highest *cis*- (68.67 %) and *trans*- (8.23 %) lycopene bioaccessibilities were obtained by the MIPEF+B+HIPEF and MIPEF+B+US+HIPEF treatments, respectively. However, the former treatment (MIPEF+B+US+HIPEF) was more effective in terms of total lycopene bioaccessibility (15.6%) in comparison to the thermally treated samples (4.95%). Hence, these findings allow for a better understanding of lycopene bioaccessibility increases in tomato juice using both thermal and non-thermal technologies; an area that has not been explored to date. Furthermore, the study quantified the negative and positive effects of each of the different processing steps on the *cis*- and *trans*- lycopene contents as well as their *in vitro* bioaccessibilities. These findings will be of value to the tomato juice processing industry where measures need to be taken in order to enhance the nutritional and functional quality attributes, apart from sensory and safety parameters.

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Conflict of interest statement

The authors declare no conflict of interest.

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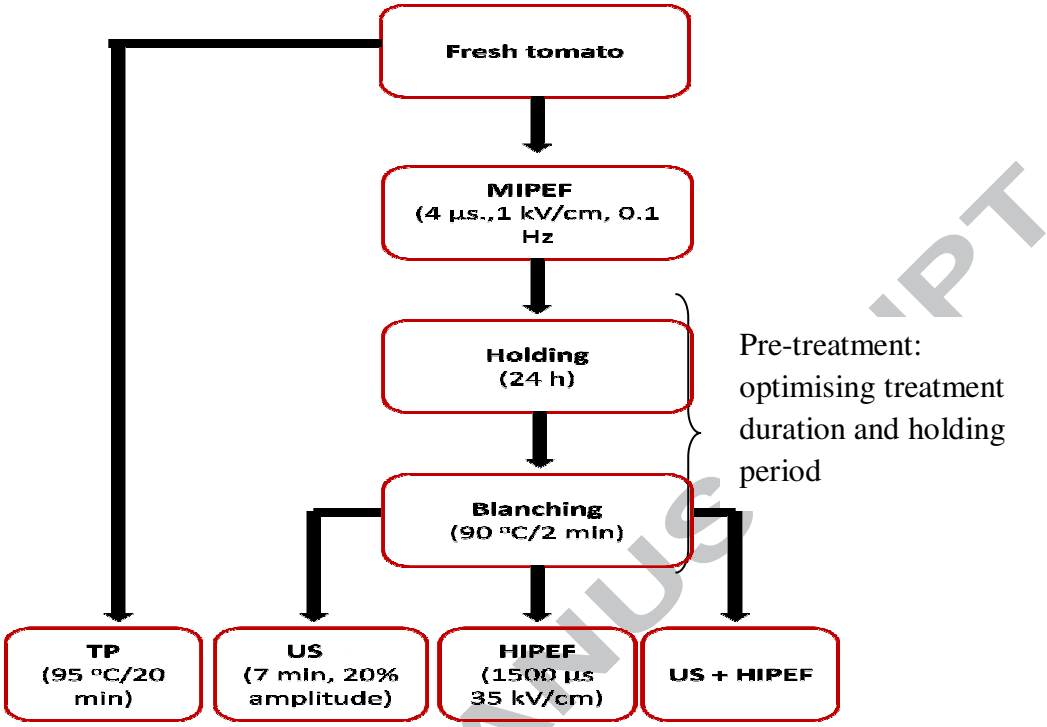

Figure 1. Overview of the experimental design for pre-treatment and full processing trials. MIPEF- Moderate Intensity Pulsed Electric Field, TP-Thermal Processing, US-Ultra Sonic and HIPEF- High Intensity Pulsed Electric Field.

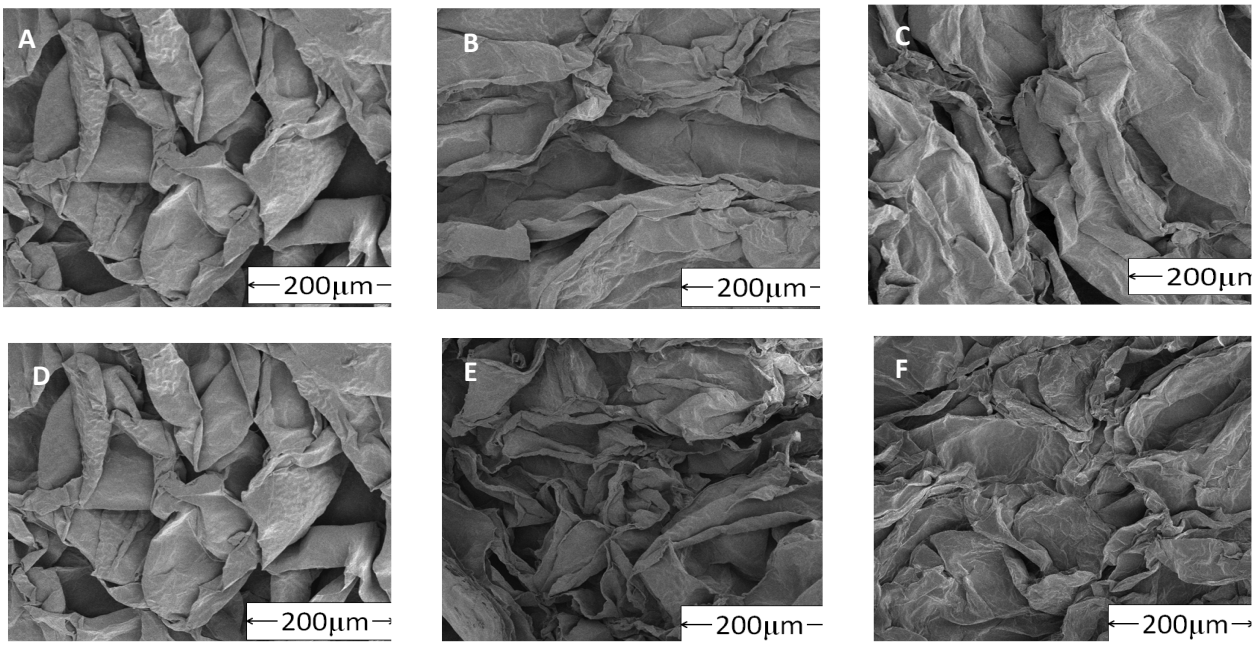

Figure 2. Effect of MIPEF treatment duration and holding period on microstructure of tomato mesocarp as shown by SEM studies . Fresh- 0 h (A), 24 h (B), 48 h (C) and 4 µs- 0 h (D) 24 h (E), 48 h (F).

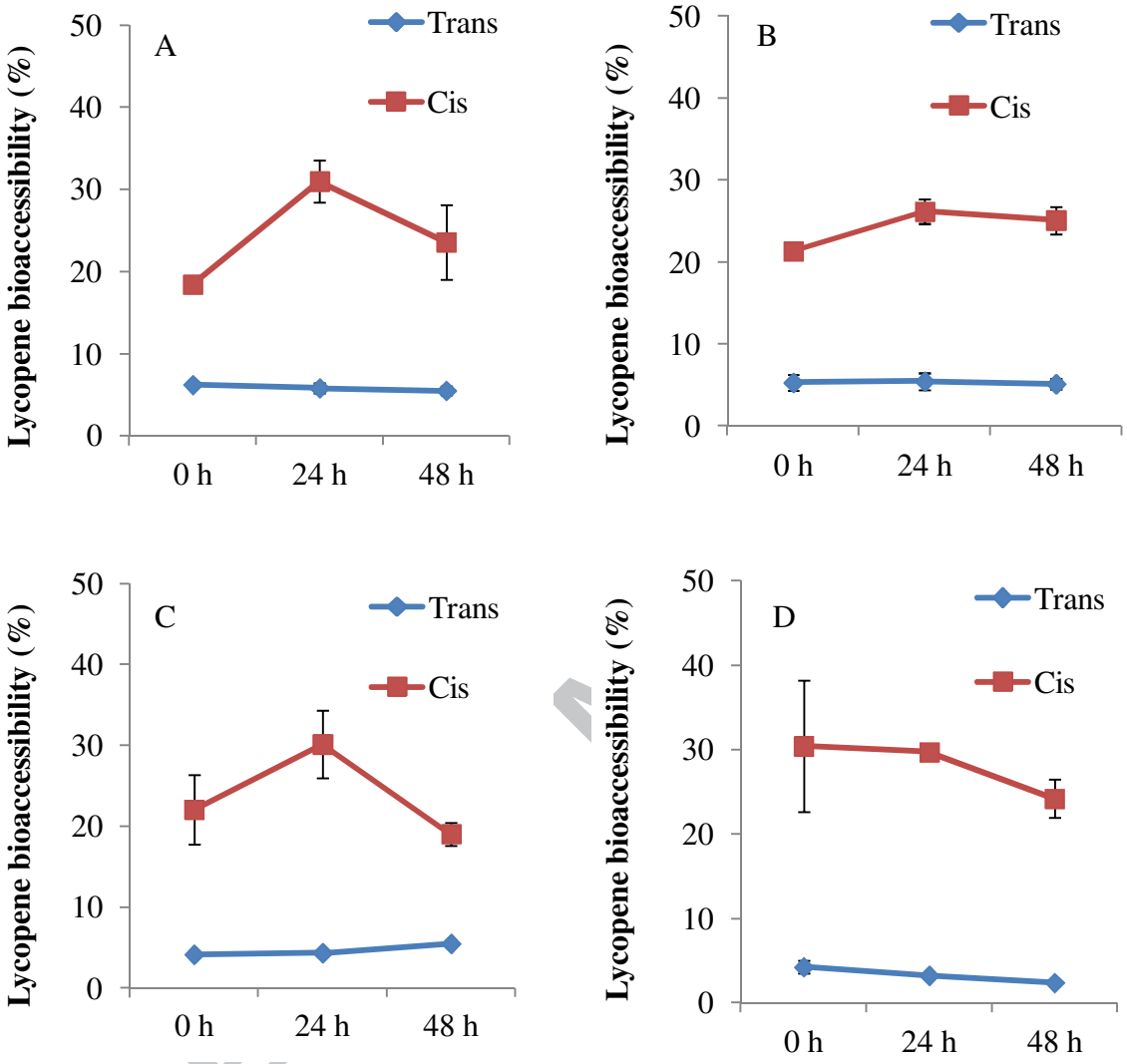

Figure 3. Effect of MIPEF treatment and holding period on *cis*- and *trans*-lycopene bioaccessibility of tomato fruits. Fresh tomato (A), 4 µs (B), 80 µs (C), 320 µs (D).

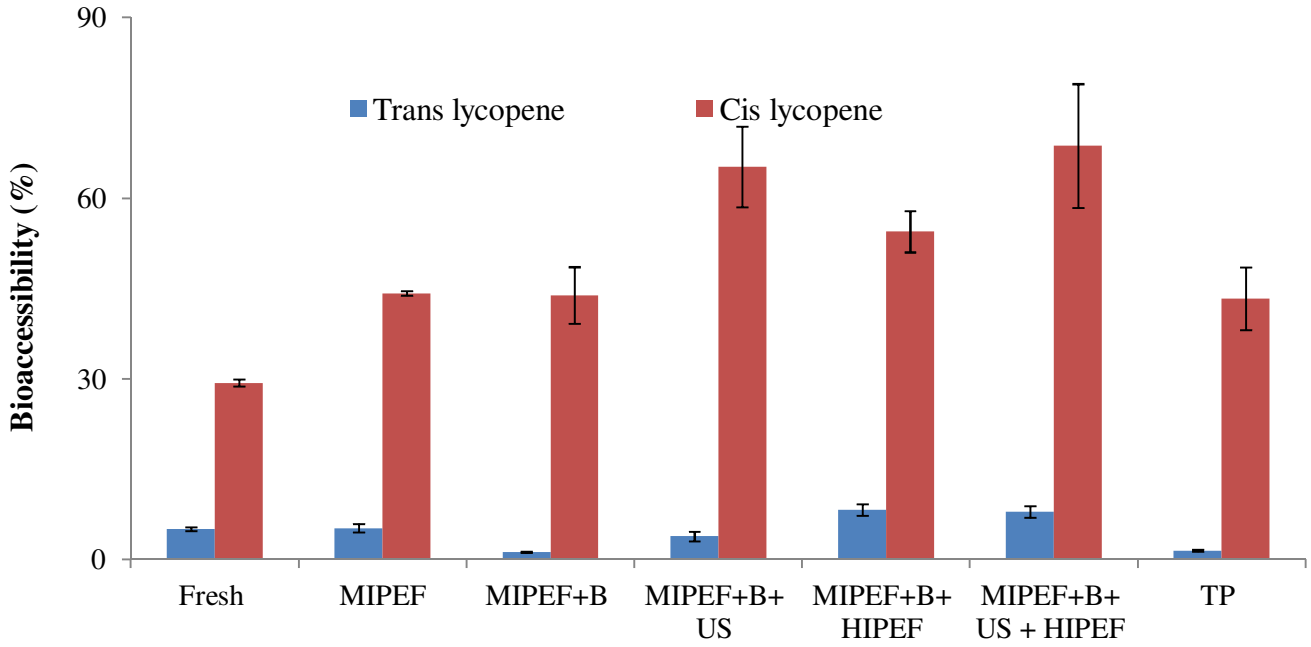

Figure 4. Effect of different processing steps on *cis*- and *trans*- lycopene bioaccessibility of tomato juice.

Table 1. Impact of PEF treatment and holding period on *trans*- and *cis*- lycopene concentration of whole tomato (n=3).

Treatment	Holding period (h)	Amount present in undigested sample			Amount present in digesta		
		Total lycopene (µg/g)	*All-trans*-lycopene (µg/g)	*Cis*-lycopene (µg/g)	Total lycopene (µg/g)	*All-trans*-lycopene (µg/g)	*Cis*-lycopene (µg/g)
Fresh	0	30.68±0.81f	25.98±0.90cd	4.70±0.32c	2.37±0.21j	1.38±0.20hi	1.00±0.06h
4 µs	0	32.53±2.68e	28.16±2.61cd	4.37±0.13c	2.54±0.16i	1.74±0.18fg	0.80±0.03i
80 µs	0	40.80±3.67dc	35.03±3.21b	5.77±0.46b	2.70±0.20i	1.44±0.09h	1.26±0.21g
320 µs	0	57.98±4.48a	50.57±3.77a	7.41±0.69a	4.34±0.60g	2.13±0.24e	2.22±0.39d
Fresh	24	29.40±1.94f	24.84±2.08d	4.55±0.21c	2.53±0.11ij	1.34±0.13hi	1.19±0.19g
4 µs	24	34.54±2.42e	29.18±1.96cd	5.36±0.47b	3.33±0.13h	1.68±0.10fg	1.65±0.04e
80 µs	24	44.38±3.51bc	38.65±3.30b	5.73±0.49b	3.37±0.17h	1.66±0.11g	1.71±0.08e
320 µs	24	45.97±3.24b	39.20±2.72b	6.77±0.55ab	3.27±0.22h	1.27±0.12i	2.01±0.13d
Fresh	48	32.35±2.18e	27.58±2.10cd	4.76±0.45c	2.61±0.16i	1.41±0.07h	1.20±0.10g
4 µs	48	38.39±1.30d	32.76±1.26bc	5.63±0.33b	3.11±0.41h	1.79±0.22f	1.32±0.19fg
80 µs	48	45.18±2.89bc	39.69±2.59b	6.49±0.30a	3.28±0.24h	2.05±0.25e	1.23±0.04g
320 µs	48	42.56±0.42c	36.35±0.31b	6.21±0.13ab	2.37±0.26j	0.87±0.11j	1.50±0.17f

Values are means ± standard deviation. Values within the same column followed by the same letter are not significantly different at $p<0.05$.

Table 2. Effect of processing step on changes in *trans*- and *cis*-lycopene concentration of tomato juice (n=3) before and after digestion.

Treatment	Amount present in undigested sample			Amount present in digesta		
	Total lycopene (µg/g)	*All-trans*-lycopene (µg/g)	*Cis*-lycopene (µg/g)	Total lycopene (µg/g)	*All-trans*-lycopene (µg/g)	*Cis*-lycopene (µg/g)
Fresh juice	29.58±1.51e	25.90±1.78c	3.68±0.30c	2.38±0.08f	1.30±0.02c	1.08±0.16c
MIPEF (M)	32.20±0.32d	28.32±0.23de	4.88±0.32b	3.63±0.07d	1.48±0.21c	2.16±0.16b
M + blanching (B)	41.49±1.78b	37.64±1.33c	3.86±0.45c	4.14±0.01c	0.46±0.04d	1.68±0.04c
M+B+US	43.43±3.41b	41.80±3.52b	1.63±0.16e	2.65±0.22e	1.59±0.23c	1.06±0.02d
M+B+HIPEF	58.02±1.69a	48.75±1.68a	9.27±0.36a	9.05±0.57a	4.01±0.48a	5.04±0.26a
M+B+US+HIPEF	37.88±2.76c	35.03±2.54c	2.85±0.32d	4.69±0.10b	2.75±0.15b	1.94±0.20bc
Thermal processing	33.84±1.12d	31.01±1.41d	2.82±0.31d	1.67±0.05g	0.46±0.04d	1.21±0.09d

Values are means ± standard deviation. Values within each column followed by the same letter are not significantly different at $p<0.05$.

HIGHLIGHTS

- Impact of processing methods on tomato lycopene bioaccessibility studied.
- Pulse electric field treatment enhanced the lycopene bioaccessibility of tomato.
- Blanching & thermal processing reduced lycopene release during digestion.
- Combined thermal & non-thermal treatments enhanced lycopene bioaccessibility.